PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

WRITTEN OPINION
(PCT Rule 66)

Date of mailing *(day/month/year)*	02.04.2004
Applicant's or agent's file reference: E-1712/03	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/05993	10.06.2003	07.06.2002

International Patent Classification (IPC) or both national classification and IPC
C12N13/00

Applicant
IGEA S.R.L. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 07.10.2004

Name and mailing address of the international preliminary examining authority:

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I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-16 as originally filed

Claims, Numbers

1-16 as originally filed

Drawings, Sheets

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-16 No
Inventive step (IS)	Claims	1-16 No
Industrial applicability (IA)	Claims	

2. Citations and explanations

see separate sheet

The following documents (D) are referred to in this opinion; the numbering will be adhered to the rest of the procedure:

D1: WO-A-0181533
D2: WO-A-0107583
D3: WO-A-0107584
D4: WO-A-0107585

1. The subject matter of claims 1-16 is not novel (Article 33(2) PCT).

1.1 D1 (abstract; page 2, lines 15-20) describes methods for electroporation including measuring the impedance ("mathematical combination (GT) of current ....and voltage" according to claim 1), i.e the ratio of the current and the voltage, calculating an objective value of a signal on the basis of the predetermined impedance and supplying the substrate with a precalculated objective value Vo ("stimulating signal", "controlled manner" according to claim 1). The electrical field applied must be high enough to permeabilize the cell membrane, but not so high as to cause irreversible damage to the cell.

D2 (abstract; Page 29, lines 13-24; claims 1-4) describes methods of controlled electroporation by passing a current across a medium comprising a cell part of a tissue. Electrical parameters may be current, voltage, impedance or a combination of current and voltage, which are used for determining the character of the cell and effect a membrane of the cell. An alternative method for detecting cells with damaged membranes would be to measure current-voltage relations across the cell, which produce immediate information. One possibility would be to use an electroporation chip for the process of electroporation. The measure of damage would be to measure the difference between an intact cell impedance and a damaged cell impedance as illustrated in Figure 8a and 8b ("waveform" according to claims 1 and 16). Thus, by modulating the amount of electrical current ("stimulating signal", "instantaneous value of the current", "controlling means" according to claims 1 and 16) it is possible to obtain electroporation without significant damage to the cell thereby obtaining a reversible situation.

D3 (abstract; page 18, lines 25 to 33; Figs 8a and 8b) describes controlled electroporation and mass transfer across cell membranes, by monitoring the

electrical impedance, which detects the onset of electroporation. The amount of electrical current can be modulated ("stimulating signal", "instantaneous value of the current", "controlling means" according to claims 1 and 16) to obtain electroporation without damaging the cells.

D4 (abstract; page 28, lines 28 to 34; page 29, lines 1-5; Figs 8a and 8b) describes controlled electroporation and mass transfer across cell membranes, by monitoring the electrical impedance, which detects the onset of electroporation. The amount of electrical current can be modulated ("stimulating signal", "instantaneous value of the current", "controlling means" according to claims 1 and 16) to obtain electroporation without damaging the cells.